

AD-A038 736

ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/13
ARENAVIRUS CONCENTRATION BY MOLECULAR FILTRATION.(U)
MAR 77 J D GANGEMI, E V CONNELL, B G MAHLANDT

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From The U. S. Army Medical Research Institute of Infectious Diseases

Frederick, Maryland

Running title: Arena virus Concentration

(9) Interim rept.

DDC
APR 28 1976
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(12) 12p.

(11) 24 March 1977

Approved for public release; distribution unlimited

AD NO.
DDC FILE COPY

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Arenavirus Concentration by Molecular Filtration

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APPROVAL		
410	DATE	BY
510	DATE	BY
610	DATE	BY
NOTIFICATION		
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DISSEMINATION/AVAILABILITY STATE		
DATE		
AVAIL. STATE		
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ABSTRACT

Liter volumes of a human arenavirus pathogen (Machupo) and a nonpathogen (Tacaribe) were concentrated 30 to 100 times in less than 90 min without significant loss of particle infectivity.

Various molecular weight polyethylene glycols have been used in the concentration of several arenaviruses (2, 7), and other animal and bacterial viruses (1, 5, 8, 11). Under ordinary laboratory conditions this method is quite satisfactory; however, under the rigorous containment conditions required for virulent viruses this method is cumbersome, time consuming, and therefore, less desirable. In addition, even small amounts of polyethylene glycol are undesirable in virus concentrates prepared for human vaccine studies. To circumvent these problems, we have utilized a molecular filtration unit manufactured by the Millipore Corporation (Pellicon Cassette System, Bedford Mass.). Virus concentration by molecular filtration has been previously described (3, 4, 9). While numerous system designs are available, the two commonly used basically consist of multiple or single membranes through which fluid flows in a recirculating or nonrecirculating mode. We have utilized both designs in our attempts to concentrate liter volumes of several arenaviruses and have found the recirculating multiple membrane Pellicon Cassette System superior, due to the speed of concentration and efficiency of virus recovery. Because of these features and the ease with which this system can be handled in a P-4 containment facility, much of the work involved in the concentration of human arenavirus pathogens can be eliminated. Figure 1 illustrates the Pellicon Cassette unit and the concentration design used in these studies.

Cell culture fluids containing $10^7 - 10^8 \log_{10}$ plaque forming units (PFU) and $10^8 - 10^9$ physical particles per milliliter of Tacaribe or Machupo virus were prepared by infecting baby hamster kidney cells (BHK-21) grown in 1/2-gallon roller bottles with the Malale or Carvalho strain of Machupo virus and strain TRVL 11573 of Tacaribe virus. The

multiplicity of inoculation for Machupo virus was 1.0 while that for Tacaribe virus was 0.1 PFU/cell. Supernatant fluids containing the virus suspended in maintenance medium E-199 (Grand Island Biologics) and 5% fetal calf serum were harvested 48-72 h postinoculation and stored at -70°C . When sufficient volumes (3 to 6 liters) of these supernatants were obtained, they were thawed and pooled prior to concentration. The supernatants were then poured into holding tank A of our concentration unit, clarified of extraneous cellular debris by filtration through two Millipore pre-filter pads contained in B and collected in holding tank C (Fig. 1A). The clarified fluid was pumped through the intake port of the molecular filtration unit D which contained five 225 cm^2 Pellicon membrane filters with 10^6 retention capacity, layered on top of one another and sandwiched between two leucite blocks (Fig. 1B). Fluid entering the unit passed over these filters (from right to left) in a tangential flow. Components with a molecular weight larger than 10^6 were presumably retained while those with a molecular weight less than 10^6 passed through the filters. These retentates and filtrates were collected separately by a manifold system contained within the filtering apparatus. The retentate was recirculated into holding tank C while the filtrate was collected separately in tank E. Flow rates were determined by the viscosity of the sample, the number and retention capacity of filters used, and the pressure applied to the recirculating fluid. Flow rates with viscous concentrates and five filters at 10 lb/in^2 averaged 60 ml/min. Table 1 lists the results from several different concentration runs with both Tacaribe and Machupo viruses.

The results illustrate the ease and efficiency of arenavirus concentration afforded by the Pellicon Cassette System. Of significance

was the fact that, even under minimal pressures, 30 to 100-fold concentrations were obtained in less than 2 h without significant loss of virus infectivity. With its high flow rate and gentle action, this system is well suited for the concentration of fragile viruses and also for the concentration of viral antigens to be used as vaccines in humans.

LITERATURE CITED

1. Albertsson, P. A. 1967. Two-phase separation of viruses, p. 303-321. In K. Maramorosch and H. Koprowski (ed.), Methods in virology, Vol. II. Academic Press Inc., New York.
2. Gschwender, H. H., M. Brummund, and F. Lehmann-Grube. 1975. Lymphocytic choriomeningitis virus. I. Concentrations and purification of the infectious virus. J. Virol. 15:1317-1322.
3. Klein, F., B. G. Mahlandt, H. B. Bonner, and R. E. Lincoln. 1971. Ultrafiltration as a method for concentrating Rift Valley fever virus grown in tissue culture. Appl. Microbiol. 21:758-760.
4. Kostenbader, K. D., Jr., and D. O. Cliver. 1973. Filtration methods for recovering enteroviruses from foods. Appl. Microbiol. 26:149-154.
5. McSharry, J., and R. Benzinger. 1970. Concentration and purification of vesicular stomatitis virus by polyethylene glycol "precipitation." Virology 40:745-746.
6. Monroe, J. H., and P. M. Brandt. 1970. Rapid semiquantitative method for screening large numbers of virus samples by negative staining electron microscopy. Appl. Microbiol. 20:259-262.
7. Ramos, B. A., R. J. Courtney, and W. E. Rawls. 1972. Structural proteins of Pichinde virus. J. Virol. 10:661-667.
8. Reddy, D. V. R., and L. M. Black. 1973. Estimate of absolute specific infectivity of wound tumor virus purified with polyethylene glycol. Virology 54:150-159.

9. Rhim, J. S., L. B. Williams, R. J. Huebner and H. C. Turner. 1969. Concentration by Diaflo ultrafiltration of murine leukemia and sarcoma viruses grown in tissue cultures. cancer Res. 29:154-156.
10. Sharp, D. G. 1965. Quantitative use of the electron microscope in virus research. Methods and recent results of particle counting. Lab. Invest. 14:831-863.
11. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology 40:734-744.

TABLE 1. Concentration of Machupo and Tacaribe viruses in a Pellicon

Cassette System

Processing conditions and results	Machupo virus		Tacaribe virus, lot		
	Malale	Carvallo	1 ^a	2	3
Time (min) ^b	60	45	90	60	90
Pressure (lb/in ²) ^c	10	10	10	25	25
Results					
Volume (ml)					
Starting	3600	2300	6000	4500	6000
Final	115	75	60	125	60
Concentration factor	31	30	100	36	100
Infectivity (PFU/ml)					
Starting	6.0×10^7	3.9×10^7	-	2.0×10^6	2.0×10^6
Final	1.7×10^9	9.0×10^8	-	9.0×10^7	1.0×10^8
Virus particles/ml ^d					
Starting	6.0×10^8	-	2.5×10^8	-	-
Final	1.7×10^{10}	-	2.0×10^{10}	-	-
% Recovery ^e					
PFU	90	75	-	100	50
Vp	91	-	80	-	-

^aFormalin-inactivated^bTotal time required for indicated concentration.^cAverage pressure for run.^dDetermined by quantitative electron microscopy (6, 10).^e(Postconcentrate/preconcentrate) x 100.

Figure Legends

Figure 1a. Concentration apparatus assembled in a P-4 containment hood line. (A) crude sample holding tank, (B) tripod filter holder containing two Millipore pre-filter pads, (C) clarified fluid holding tank, (D) Millipore Pellicon Cassette Molecular Filtration unit and peristaltic pump with rate controller (not labeled). (E) filtrate holding tank.

Figure 1b. Diagramatic sketch of molecular filtration unit.

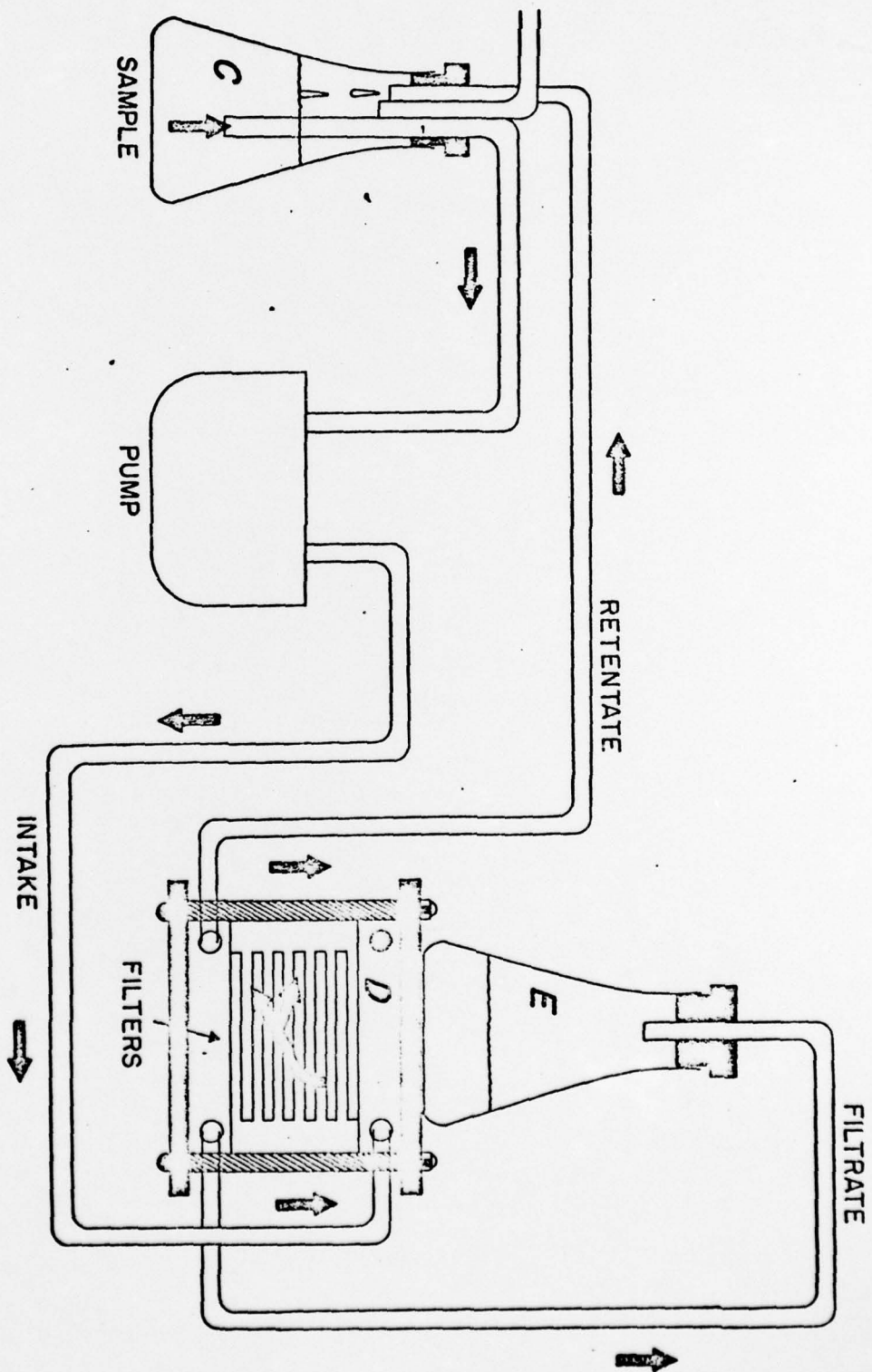


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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Arenavirus Concentration by Molecular Filtration		5. TYPE OF REPORT & PERIOD COVERED Interim
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) J. David Gangemi, Edward V. Connell, Bill G. Mahlandt and Gerald A. Eddy		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases, SGRD-UIV-I Fort Detrick, Frederick, Maryland 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS BS03-00-0004
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE 24 Mar 77
		13. NUMBER OF PAGES 10
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release, distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in the Journal of Applied and Environmental Microbiology.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Machupo, Tacaribe, Concentration, Molecular filtration		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Liter volumes of a human arenavirus pathogen (Machupo) and a nonpathogen (Tacaribe) were concentrated 30 to 100 times in less than 90 min without significant loss of particle infectivity		